Time Course of Export of ^<14>C-assimilates and Their Distribution Pattern as Affected by Feeding Time and Night Temperature in Cucumber Plants

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Time Course of Export of ^{14}C-assimilates and Their Distribution Pattern as Affected by Feeding Time and Night Temperature in Cucumber Plants

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Summary

The export and the distribution pattern of ^{14}C pulse-fed to a single leaf were investigated in cucumber plants in comparison with tomato plants in the previous report (1).

The percentage total export of ^{14}C 4 hours after feeding was about 20(%) irrespective of the feeding time, while its increase during the following 4 hours was −1 to 21(%) and larger after the later feedings. Slow export continued still thereafter, and the percentage total export amounted to 30 to 35(%) by the next morning. Thus, the time course of ^{14}C export was similar to that of tomato plants, though the percentage values were somewhat lower in cucumber plants.

With these values the ratio of the ^{14}C exported in the daytime to that during the following night was calculated at about 1:1, which did not appear to be much different from the ratio of 3:4 obtained in tomato plants. In addition, the comparatively slow rate of the increase of ^{14}C export from 4 to 8 hours after the earlier feedings seemed to be due to the incorporation of ^{14}C into the starch and the resultant reduction of the pool size of ^{14}C-sugars as translocates.

The export of ^{14}C fed in the afternoon was retarded at night temperatures of 5° and 9°C, and especially so for the plants at the 20-leaf stage. The percentage distribution to the lower parts including the roots 16 hours after feeding was comparatively higher at the 7-leaf stage, varying from 92(%) at 5°C to 62(%) at 24°C, thus, it decreased with increasing night temperature, but increased a little at 30°C. At the 20-leaf stage, it was 62 to 44(%), being highest at 12°C and decreased both below and above 12°C. The low values at 5° and 9°C were considered to be due to the delay of the export. Thus, it would be probable that there is little difference between cucumber and tomato plants not only in the time course of the export but also in the effects of night temperature on the distribution pattern.

Cucumber and tomato are the major crops under protected cultivation in Japan, and it is most important to elucidate their physiological response to the controlled environments. To know the ratio of the export in the daytime to that during the following night as well as the effect of night temperature on the

distribution pattern of the assimilates, for example, is indispensable to evaluate the practical merits of controlled night temperature. For tomato plants, we reported previously (1) that the time course of the export varied with the feeding time and the ratio of the export in the daytime to that during the following night was calculated at 3:4, which did not agree with the ratio of 3:1 reported by Toki (2) using a half leaf method. In addition, the percentage distribution to the lower parts decreased with increasing night temperature.

The present work was to determine whether or not cucumber plants were different from tomato plants in their physiological behaviour regarding the time course of the export of ^{14}C-assimilates and their distribution pattern as affected by night temperature.

Materials and Methods

Cucumber cultivar 'Natsusairaku No. 3' was used throughout the experiments.

Experiment 1. In April, 1975, seedlings were pricked out into 18-cm clay pots when the cotyledons were horizontal, and grown in a glasshouse. Prior to ^{14}C feeding, the plants were transferred in a phytotrone kept at day/night temperatures of 24°/17°C and on May 23, when they were at the 6-leaf stage, they were divided into three groups, and 20 μCi of $^{14}CO_2$ was supplied to the third leaf of individual plants of each group for 30 minutes ending at 9.00, 13.00 and 17.00 hr respectively. At 17.00 hr, all the plants were removed to 17°C in the dark and again to 24°C in the light at 9.00 hr the next morning. In each group 12 plants were used. Three plants each were sampled 4, 8, and 16–24 hours after feeding respectively as shown in Fig. 1. The plants were immediately severed into several component parts, air-dried and pulverized for the determination of ^{14}C-radioactivity.

Experiment 2. In July, 1975, 20 μCi of $^{14}CO_2$ was supplied to the third leaf of individual plants at the 7-leaf stage. Then the plants were divided into three groups, which were removed to 17°C in the dark 0, 4 and 8 hours after feeding respectively. For each group, three plants were harvested 2, 4, 8 and 24 hours after feeding respectively. They were severed into several component parts, air-dried and determined for ^{14}C radioactivity. The dried samples of fed leaves were extracted at 80°C with 80 per cent ethanol, and the extracts were analysed for total sugar by Somogyi's method. While, the residues were further extracted with perchloric acid and analysed for starch. Further, the ^{14}C in the ethanol soluble fraction (instead of that in the total sugar) and in the starch were determined, then the ratios of total sugar/starch and ^{14}C in the ethanol soluble fraction/^{14}C in the starch were calculated and expressed as R_c and R_s respectively. In addition, sugar composition and ^{14}C incorporation into sugars 30 minutes and 16 hours after feeding were investigated by paper and radio chromatographies.

Experiment 3. In April, 1976, seedlings were pricked out into 15-cm clay pots and grown in a growth chamber of 17°/12°C for a week to secure the differentiation of

female flowers on the lower nodes. Then, they were transferred in a phytotrone kept at 24°/17°C. All the axillary shoots, male flowers and tendrils were removed as they emerged. The uniform plants were used for experiment when they reached the 7- and 20-leaf stage respectively on June 1 and June 20, the latter being 3 days after the anthesis of female flower on the ninth node. Twenty μCi of $^{14}CO_2$ was supplied to the third leaf of individual plants at the 7-leaf stage and to the eighth leaf at the 20-leaf stage respectively for 30 minutes ending at 17.00 hr. Immediately after feeding, three plants each were transferred to the dark rooms maintained at 5°, 9°, 12°, 17°, 24° and 30°C, and harvested after 16 hours. The harvested plants were air-dried and counted for ^{14}C radioactivity.

General Procedures of Feeding, Determination of Radioactivity and Representation of the Results. All procedures were the same as described in the previous paper dealing with tomato plants (1), except that wet digestion by van Slyke Folch, instead of combustion by a Packard sample oxidizer, was used for the determination of ^{14}C. Twenty mg of dried material was decomposed under reduced pressure (ca. 15 mm Hg) by heating gently with 10 ml of oxidizing solution (40 g of CrO_3 was dissolved into 334 ml of H_3PO_3 and 667 ml of $H_2SO_4 \cdot 20\%$ SO_3, then the solution was heated to 160°C and cooled.). The generated CO_2 ($^{14}CO_2+^{12}CO_2$) was absorbed in 15 ml of absorbing solution (85 ml of methylecellosolve mixed with 10 ml of triethanolamine) for 2 hours, 0.5 ml of which was mixed with 10 ml of toluene scintillator, and ^{14}C radioactivity was determined using a liquid scintillation counter (Horiba-LS 500).

Results

Time Course of Export of ^{14}C-assimilates as Affected by Feeding Time.

The total export of ^{14}C from the source leaf increased rapidly and reached 18 to 23 per cent 4 hours after feeding irrespective of the feeding time. The increase of the export during the following 4 hours was larger after the later feedings, although not so large as that during the first 4 hours, and even a slight decrease was observed in the plants fed at 9.00 hr. Thus, the percentage total export 8 hours after feeding was near 35(%) in the plants fed at 17.00 hr but below 25(%) in those fed at 9.00 and 13.00 hr. Thereafter, the export continued slowly and reached 30 to 35 per cent in the next morning irrespective of the feeding time (Fig. 1).

Using the results in Fig. 1 and on the assumption that the photosynthetic rate was kept constant throughout the daytime and the respiration rate was the same all over the plant body, the total exports in the daytime (8 hours), during the following night (16 hours) and in a day were approximated with equations in relation to the feeding time as shown in Fig. 2. By integrating the equations, it was indicated that the total export in a day was 30.3 per cent, and the ratio of the export in the daytime to that during the following night was about 1:1 (Fig. 2).

In the change of the distribution pattern with the time after feeding there was not necessarily observed a consistent trend. But it would be probable that the percentage distribution to the roots was a little higher after the later feedings and some retranslocation from the roots occurred the next morning and thereafter (Fig. 3).

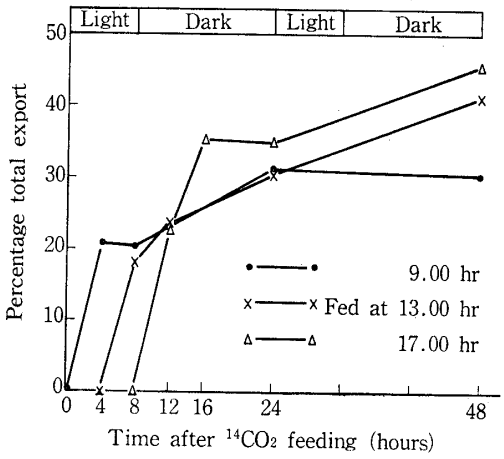


FIG. 1

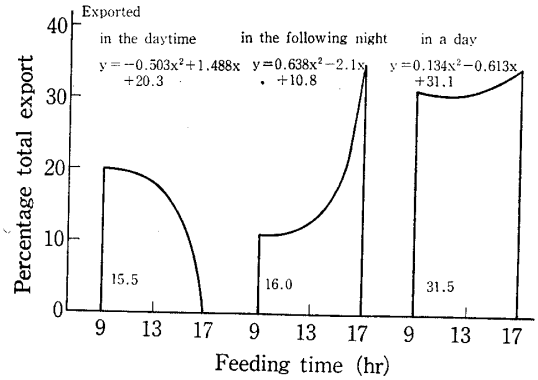
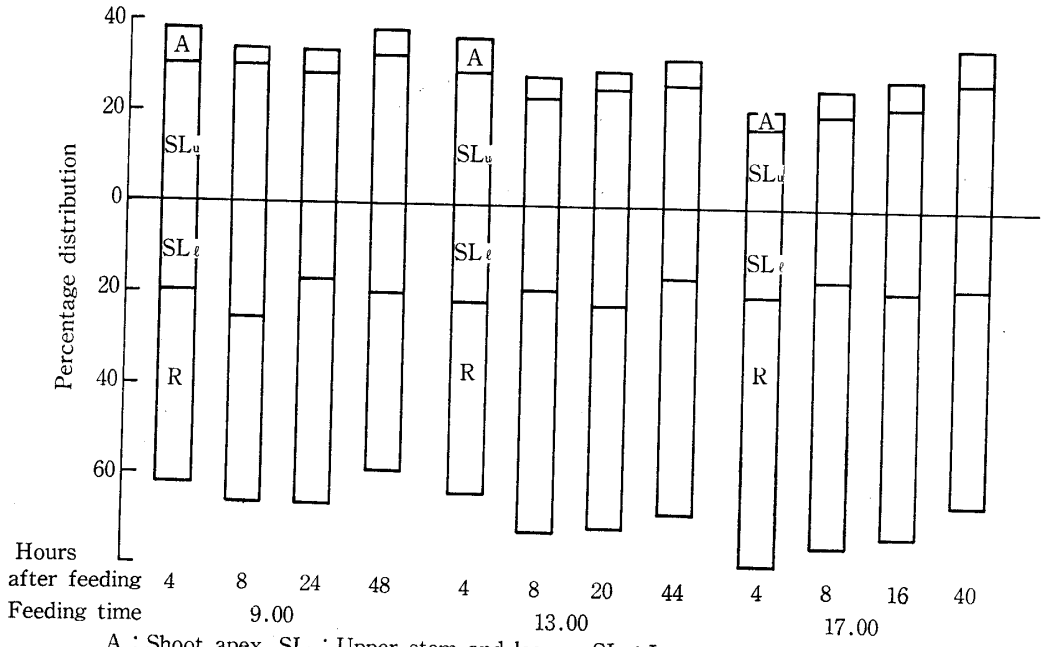


FIG. 2

FIG. 1. Time course of the total export of ^{14}C-assimilates as affected by the feeding time.
FIG. 2. Total export of the ^{14}C-assimilates in the daytime, during the following night and in a day as affected by the feeding time. y=Percentage total export, x=Feeding time (hr) − 9



A : Shoot apex, SL_u : Upper stem and leaves, SL_ℓ : Lower stem and leaves, R : Roots

FIG. 3. Distribution pattern as affected by the feeding time and varying with time after feeding.

Time Course of Export of ^{14}C-assimilates in Relation to Carbohydrate Status of Source Leaf.

The time course of the export of ^{14}C-assimilates varying with the lit hours after feeding (Fig. 4) corresponded well to that shown in Exp. 1, and it was clearly shown if the curves for the 0- and 4-hour lit plants were moved 8 and 4 hours to the right, respectively, in Fig. 4. The values for the 4-hour lit plants, however, were unreasonably low at 8 and 16 hours after feeding.

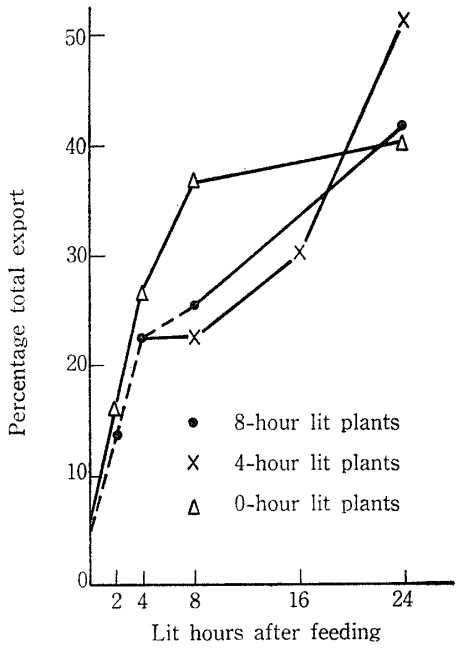


FIG. 4. Total export of ^{14}C-assimilates as affected by the time in the light after feeding.

In the source leaves of the plants removed to the dark immediately after feeding (the 0-hour lit plants), the total sugar content decreased rapidly, whereas the starch content remained at the initial level, and in consequence, the R_c value decreased rapidly, until 8 hours after feeding. Thereafter, starch began to be hydrolyzed and contribute sucrose to the translocate pool, and thus, the decrease of R_c value slowed down. In the 8-hour lit plants, however, photosynthesis was continued still after feeding, and the total sugar retained the initial level in spite of the loss through translocation from the leaves as well as through incorporation into starch within 4 or 8 hours after feeding, and then, turned to a slight decrease. While, the starch content increased rapidly during the first 4 hours, kept the high level during the further 4 hours, and then, turned to a decrease when the plants were removed to the dark. The R_c value changed with time almost in inverse proportion to the starch content. The R_a value showed the same trend with the R_c value in general, although more exaggerated because of the fact that the leaves were pulse-fed with $^{14}CO_2$ (Fig. 5 and 6).

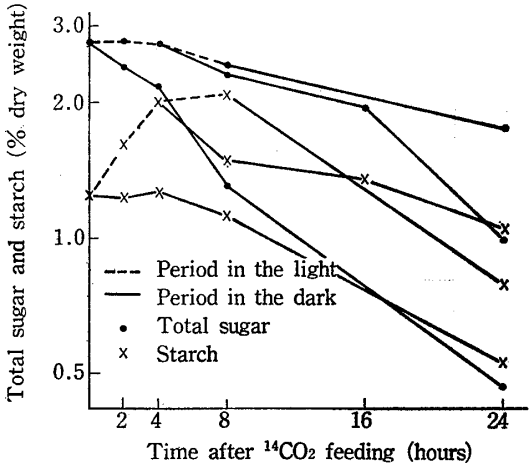


FIG. 5

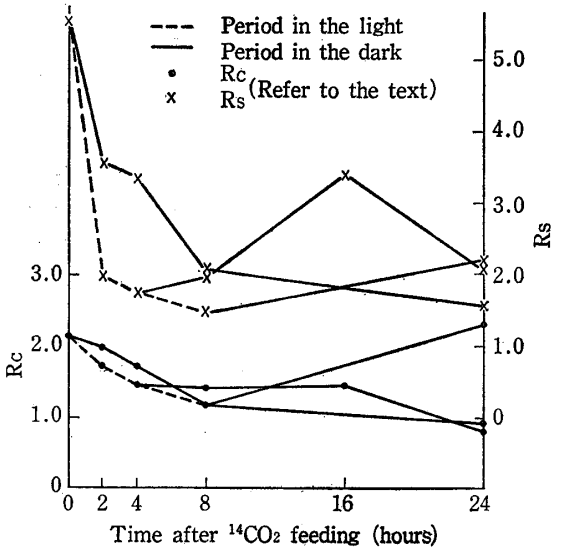


FIG. 6

FIG. 5. Changes with time of the total sugar and starch contents in the source leaves in relation to the lit hours after feeding.

FIG. 6. Changes with time of the values of R_c and R_s in relation to the lit hours after feeding.

In general, the cucumber leaves contain comparatively large quantities of fructose, glucose and sucrose but small quantities of stachyose and raffinose. In such a sugar composition there was found, as a matter of course, no difference between 30 minutes and 16 hours after feeding. While, the distribution of ^{14}C among sugars varied with the time after feeding. Thus, 30 minutes after feeding, when an active export was observed, stachyose and raffinose and next sucrose were labeled intensely but fructose and glucose only slightly with ^{14}C. Sixteen hours after feeding, when a slow export was continued, however, ^{14}C was distributed uniformly among sugars, that is, in its quantity, to hexoses, sucrose, and stachyose and raffinose in declining order (data not shown).

Translocation and Distribution of ^{14}C-assimilates as Affected by Night Temperature.

When fed at the 7-leaf stage, the total export of ^{14}C was markedly delayed at a night temperature of 5°C and the percentage total export 16 hours after feeding was 22(%) as compared with 34 to 42(%) at higher night temperatures. The distribution pattern was also affected by night temperature. The lower the night temperature, the higher the percentage distribution to the lower parts, although at 30°C it decreased a little (Fig. 7-a).

When fed at the 20-leaf stage, the percentage total export increased with night temperature, ranging from 27(%) at 5°C to 54(%) at 24°C, but decreased a little at 30°C. The percentage distribution to the lower parts decreased with increasing night temperature at and above 12°C, while it decreased at 5° and 9°C,

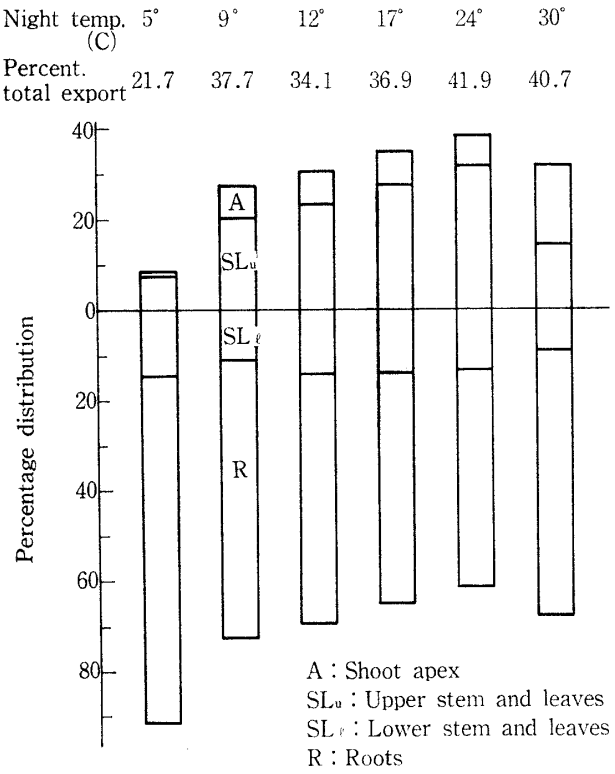


FIG. 7-a. Distribution pattern of ^{14}C-assimilates as affected by night temperature (the 7-leaf stage, 16 hours after feeding).

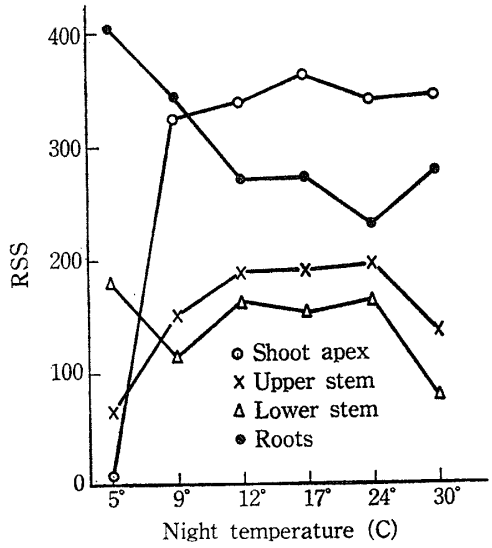


FIG. 7-b. Sink activity as affected by night temperature (the 7-leaf stage, 16 hours after feeding).

differing from at the 7-leaf stage. It should be noted, however, that at 5° and 9°C, the percentage distribution to the roots was markedly low as compared with that at higher temperatures. The percentage distribution to the fruit was lowest at 12°C and increased both below and above 12°C (Fig. 8-a).

The RSS values (Fig. 7-b and 8-b) corresponded rather well with the distribution

pattern except for some discrepancies in particulars. At the 7-leaf stage, they were low in the roots at higher temperatures except 30°C and also low in the upper parts such as the shoot apex and the upper stem at 5° and 30°C. At the 20-leaf stage, they were low in the roots but high in the lower stem, and in the fruit they were lowest at 12°C and increased both below and above 12°C.

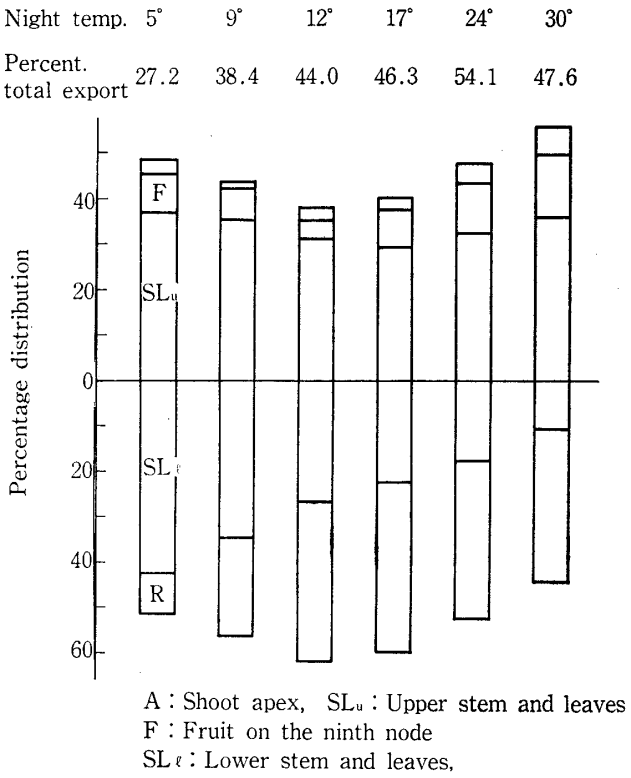


Fig. 8-a. Distribution pattern of ^{14}C-assimilates as affected by night temperature (the 20-leaf stage, 16 hours after feeding).

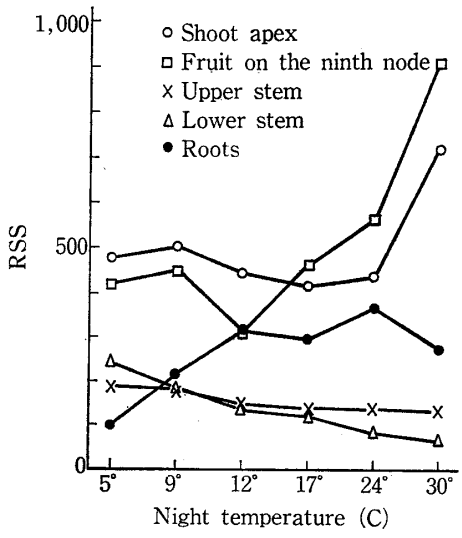


Fig. 8-b. Sink activity as affected by night temperature (the 20-leaf stage, 16 hours after feeding).

Discussions

The time course of the export of ^{14}C-assimilates obtained in cucumber plants in relation to the feeding time was almost the same with that in tomato plants reported previously (1). Thus, there were recognized a rapid phase during 4 or 8 hours immediately after feeding and a slow phase thereafter. The current export appeared to be almost completed by the next morning at a night temperature of 17°C, reaching the percentage total export of 30 to 35(%). During the latter half of the rapid phase, however, the export was slower after the earlier than the later feedings. In the earlier feedings, photosynthesis was continued after feeding and the assimilated ^{14}C was incorporated into starch, which appeared to result in the slow-down of the ^{14}C export. During the first half of the rapid phase, however, the increase of the sugar pool as a whole following the continued photosynthesis after the earlier feedings seemed to compensate for the decrease of the ^{14}C content in the sugar pool, and consequently, there seemed to be no difference due to the feeding time in the increasing rate of the ^{14}C export. The same phenomena were already reported in potatoes (3). With darkness, however, the ^{14}C-starch began to contribute ^{14}C-sucrose, and perhaps not ^{14}C-stachyose and -raffinose, to the translocate pool through hydrolysis. Thus, during the slow export in the night, ^{14}C was exported mainly as ^{14}C-sucrose, and in comparatively larger quantities after the earlier than the later feedings. Geiger (4) described in beets that during the early part of the dark period sucrose reserves in the supply leaf serves as the major source of translocate, while after 2 to 3 hours supply leaf starch-like polysaccharide reserves began to replenish the sucrose pool.

The ratio of the export in the daytime to that during the following night was calculated at about 1:1, though with some reservations. This value was a little higher than the ratio of 3:4 reported previously in tomato plants (1), but there seemed to be no fundamental difference between the two crops. While, Toki (2) reported the ratios of 1:3 and 3:1 respectively for cucumber and tomato plants using a half leaf method. Such discrepancies in the ratio values between the investigators should be further examined from a methodological viewpoint. Hori and Arai (5) and Kamota and Naito (6) reported the trend of cyclic growth of the shoot and fruit in cucumber plants, that is, their elongation rate was comparatively high around midnight, and such a trend was no less marked than in tomato plants (5, 6, 7). These facts, however, do not necessarily imply that the assimilates are exported to the meristematic tissues exclusively in the night, as pointed out by Baker and Moorby (3) for the growth of potato tubers.

The percentage distribution of ^{14}C-assimilates to the roots was higher after the later feedings as in tomatoes, sweet potatoes, beets, etc. (8, 9, 10), the fact of which proves that the uptake of the assimilates into the roots is most vigorous in the dark. Moreover, the decrease of the percentage distribution to the roots with time after feeding was more clearly shown than in tomato plants, and it began earlier

after the later feedings, though it was only 10 per cent at most. Such a decrease appeared to be due to the occurrence of the retranslocation of the assimilates having once accumulated in the roots.

The velocity of the export was, of course, influenced by temperature. Thus, the export of ^{14}C assimilated in the evening was remarkably delayed at a night temperature of 5°C, and at 9°C it was delayed a little only in the plants at the 20-leaf stage. In such cases, the percentage distribution to the lower parts, and especially to the roots was remarkably low, but expected to increase with time. With such exceptions, the lower the night temperature, the higher the percentage distribution to the lower parts, and it was almost the same as in tomato plants, although Yoshioka *et al.* (11) obtained opposite results to ours by feeding a whole plant in tomatoes. The high values of the percentage distribution to the fruit at 5° and 9°C, however, could not be well explained.

The importance of the distribution pattern as affected by night temperature was discussed in the previous paper (1) in relation to the environmental control in the protected cultivation. It would be possible to conclude from this study and also from the previous paper dealing with tomato plants (12), that air temperature, rather than root temperature, in the night is the major factor that determines the distribution pattern. The retranslocation of the ^{14}C-assimilates having been once accumulated in the roots will be reported for tomato plants in another paper.

It should be well noted that the figures of the percentage total export and the percentage distribution in this paper are somewhat different from those presented at the Annual Meeting of the Japanese Society for Horticultural Science in 1975 (pp. 232–5 in the Proceedings), because in the latter ^{14}C found in the petiole of the fed leaf after feeding was considered to have been exported, whereas in this report, not to have been exported, as in the tomato experiment reported previously, where it was difficult to distinguish between the leaf blade and petiole.

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